UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

**DRAFT DELIBERATIVE**

**MEMORANDUM**

**SUBJECT:** Environmental Risk Assessment of OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034) Protein, a DsRed2 Protein Variant (DsRed2-OX5034), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 *Aedes aegypti*; Data and Information Were Provided in Support of an Extension and Amendment to an Experimental Use Permit for Release in California and Florida.

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|---|---|
| **Parent Code Case:** | 00295568 |
| **Action Code Case:** | 00295569 |
| **EPA File Symbol:** | 93167-EUP-2 |
| **MRID Number:** | 51361701 |

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**TO:** Matt Weiner, Risk Manager
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## I. BACKGROUND AND EXECUTIVE SUMMARY

Oxitec Ltd., (Oxitec) currently holds an Experimental Use Permit (EUP), which is authorized only in the states of Florida and Texas, to evaluate efficacy of the release of OX5034 *Ae. aegypti* mosquitoes against wild *Aedes aegypti* mosquitoes (hereinafter referred to as *Ae. aegypti* mosquitoes) within Monroe County, Florida and Harris County, Texas. Several key factors played a significant role in the previous assessment and determination that OX5034 *Ae. aegypti* male mosquitoes would not result in adverse effects for humans or the environment as a result of the approved experimental permit. Below are the key factors as outlined in the previous risk assessment [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>558</RecNum><DisplayText>(USEPA 2020b)</DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629394477">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti; Data and Information Were Provided in Support of a FIFRA Section 5 Application. Memo from Wiebke Striegel and Amanda Pierce to Eric Bohnenblust, dated April 30, 2020.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]:

- Only male OX5034 mosquitoes will be released into the environment. Because male mosquitoes do not feed on humans (they do not bite), they do not pose a human health risk.
- Female mosquitoes feed on human blood, but only once they become adults.
- Oxitec's OX5034 female mosquitoes do not survive to become adults without tetracycline. Tetracycline acts as an antidote to the OX5034 female mosquito-lethal trait.
- EPA evaluated penetrance of the OX5034 female-lethal trait.
  - Penetrance for the OX5034 mosquitoes refers to the proportion of female insects that die before reaching adulthood, i.e., does it consistently work. EPA found that it does.
- EPA evaluated human health risk of OX5034 mosquitoes.
  - A determination of the toxicity and allergenicity of the two substances in Oxitec's OX5034 mosquitoes that 1) kill female mosquitoes, tTAV-OX5034, and 2) allow trained personnel to identify OX5034 via fluorescence, DsRed2-OX5034, has not been made.
  - However, because no OX5034 female mosquitoes are being released or are expected to emerge in the environment, exposure is negligible and therefore, so is the potential risk from tTAV-OX5034 and DsRed2-OX5034 (Risk = Exposure x Hazard).
- EPA evaluated introgression risk.
  - Introgression for the OX5034 mosquitoes refers to the movement of background traits from the non-GE portion of the OX5034 mosquito genome to local mosquitoes, i.e., will releases of OX5034 mosquitoes increase the ability of wild mosquitoes in the release area to vector/transmit disease, result in larger populations numbers, or result in more

**Commented [WBJ1]:** Can we mention here the docket where this can be found?

**Commented [WBJ2]:** This language is awkward, and I'm not about punctuation. Can this be stated more formally? Maybe something like: "That is, 'penetrance' is a measure of the consistency of efficacy." But that's problematic, in that "efficacy" is what the EUP is supposed to necessary to evaluate. If it's already been found to be "efficacious," then an EUP is not appropriate and they should be applying for a section 3 registration.

...After further reading, I think this just needs better explanation of what you mean. I think you mean that "penetrance" evaluates how well the "kill switch" in females works, and ***not*** how well the overall product works in reducing mosquito populations (which is the point of the EUP). If that's correct, then can you state that more clearly?

**Commented [WBJ3]:** Same comment as above. Can you be more specific about what, exactly EPA found? Again, I don't think it can be that EPA has already found the product to be "effective," because that is the sole purpose of the EUP, and if the EUP is not needed for that, then Oxitec should be seeking a section 3 registration.

**Commented [WBJ4]:** Should this be "population"?

robust mosquitoes. EPA found that these impacts are unlikely. As part of this analysis, EPA collaborated with the United States Centers for Disease Control and Prevention (CDC) in reviewing laboratory data, a meta-analysis, and rationale submitted by the applicant comparing the vectorial capacity of OX5034 mosquitoes to that of wild mosquitoes.

- EPA evaluated the risk of OX5034 mosquitoes to non-target organisms (bats, amphibians, etc.).
  - No direct adverse effects due to consumption of OX5034 males by non-target organisms is expected based on acute oral toxicity studies and bioinformatics analyses.
  - *Ae. aegypti* mosquitoes (including theof which OX5034 mosquitoes are) are not a sole or critical food source for non-target organisms, so no indirect adverse effects are expected should there be a decrease in the local mosquito population.

Based on the above factors and analyses discussed in EPA's science assessment for the previously granted EUP [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>558</RecNum><DisplayText>(USEPA 2020b)</DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629394477">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti; Data and Information Were Provided in Support of a FIFRA Section 5 Application. Memo from Wiebke Striegel and Amanda Pierce to Eric Bohnenblust, dated April 30, 2020.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], EPA determined that there would be no adverse effects to humans or the environment as a result of the experimental use permit to release Oxitec's OX5034 male mosquito. Under the current action, Oxitec requests a 24-month extension of its experimental use permit in Monroe Co., FL and requests additional testing locations for twelve California counties. The requests under the proposed extension and amendment do not alter the risk conclusions of the original EUP. Based upon an analysis of the application for extension and amendment of the EUP, below are EPA's risk conclusions for the proposed EUP extension and amendment, which can also be found in Unit IV, "Environmental Risk Conclusions:"

EPA concluded that the potential of *exposure* of any nontarget organisms, which includes endangered and threatened species, to OX5034 *Ae. aegypti* male mosquitoes is limited due to species-specific behavioral traits of *Ae. aegypti* resulting in its preferential habitat being largely limited to areas surrounding human dwellings and its preferential breeding sites being largely composed of man-made containers.

EPA concluded that the consumption of OX5034 *Ae. aegypti* male mosquitoes by nontarget organisms is not expected to pose a hazard to any nontarget organisms, which includes endangered or threatened species, based on 1) bioinformatics analyses demonstrating lack of similarity between DsRed2-OX5034 or tTAV-OX5034 and known toxins, 2) bioinformatics analyses demonstrating susceptibility of DsRed2-OX5034 or tTAV-OX5034 to gastric proteases, 3) toxicity study indicating no adverse effects to fish upon OX5034 *Ae. aegypti* male mosquito consumption, and 4) toxicity study indicating no adverse effects to an aquatic invertebrate upon OX5034 *Ae. aegypti* male mosquito consumption.

EPA concluded that the possible reduction of the *Ae. aegypti* populations in the EUP locations is not expected to pose a hazard to any nontarget organisms, which includes endangered or threatened species, based on 1) literature reviews that indicate that no species are reliant on *Ae. aegypti* mosquitoes as a food source, 2) the generalist nature of predators that consume mosquitoes, 3) species-specific behavioral traits

**Commented [WBJ5]:** Should this be a question mark? Alternatively, can this be stated less like a question?

**Commented [WBJ6]:** Is "unlikely" the exact term used in the RA that this is citing?

**Commented [WBJ7]:** For all of the remaining paragraphs in this section, it's not clear whether you are talking about the previous/existing EUP, or the Amendment/Expansion application (??).

**Commented [WBJ8]:** Is this sentence referring to the previous/existing EUP, or this amendment/expansion application?

**Commented [WBJ9]:** Why? Is this discussed in detail below?

**Commented [WBJ10]:** And Unit V, "Risk to Federally Listed Threatened and Endangered Species," right?

**Commented [WBJ11]:** You're talking about the current amendment/extension application, correct? So should this be "concludes?" If that's correct, it might be more clear to start each of these paragraphs with something like "Regarding the proposed EUP extension and amendment…"

**Commented [WBJ12]:** I've italicized this word in order to make clear that the "limited" finding only goes to "exposure," and not "effect," because if the "effect" on listed species was merely "limited," that sound like a "may affect" which would require consultation with the Services (informal or formal, depending on NLAA or LAA).

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**Commented [WBJ13]:** Same comment.

**Commented [WBJ14]:** Same comment.

of *Ae. aegypti* that limit the potential for interaction with nontarget organisms, 4) the invasive species status of *Ae. aegypti* which reduces the likelihood that any significant co-evolutionary relationships exist with nontarget organisms in the United States, and 5) *Ae. aegypti* is commonly targeted for pest reduction through mosquito control measures which further limits the likelihood that a nontarget organism would be reliant upon this species for food.

Therefore, although exposure may be possible (but is expected to be limited), and because no hazard was identified (i.e., no hazard from oral consumption or from the reduction of the local *Ae. aegypti* population), EPA concluded that there is a reasonable expectation of no discernible effects for nontarget organisms as a result of the experimental use permit to release OX5034 *Ae. aegypti* male mosquitoes. Therefore, since no discernible effects are anticipated to any nontarget organism, a "No Effect" determination is also made for direct and indirect effects to federally listed endangered and threatened species, and for their designated critical habitats.

**Commented [WBJ15]:** Same comment.

**II. Introduction**

Oxitec requests an amendment and extension of its existing EUP under FIFRA section 5 for the end-use product OX5034 containing a variant of the active ingredient tetracycline-repressible transactivator (tTAV-OX5034) protein, a variant of the inert ingredient DsRed2 protein (DsRed2-OX5034), and the genetic material (vector pOX5034) necessary for their production in OX5034 *Ae. aegypti*.

Under the EUP extension and amendment, Oxitec requests to continue to test the efficacy of the product by deploying OX5034 mosquito eggs and adult males in the treatment areas. Importantly, as described in USEPA 2020, only male OX5034 mosquitoes would emerge from these eggs and be released, and no female OX5034 mosquitoes would be released. Specifically, the request is for a 24-month permit for a cumulative area up to 90,840 acres, which would be divided into multiple treatment and control areas within Monroe, Co., Florida and twelve possible counties in California (Shasta, Stanislaus, Alameda, Sacramento, Yolo, Fresno, Kings, Tulare, Los Angeles, Orange, San Bernardino, Riverside Counties; Figure 1).

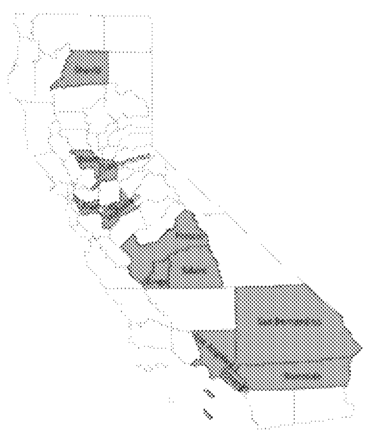

**Figure 1.** Twelve California counties proposed for field testing.

## A. Approach

Modified mosquito release products by their very nature – i.e., biological substances produced and used in living mosquitoes – present different considerations than other types of pesticides when considering appropriate data requirements for risk assessment. EPA refers to the biochemical and microbial data requirements listed at 40 CFR part 158, which are used to register a biopesticide, to determine appropriate data needs for a modified mosquito release product. A data requirement identified as appropriate for a modified mosquito release product can be met through generating the data identified in the data requirement, including through the use of alternative species, or in lieu of generating such data by submitting or citing results from previously conducted studies, and/or citing publicly available literature. When relying on information sources in lieu of empirical data generation, a rationale should be submitted explaining how the cited literature and already available data are sufficient to allow the Agency to assess the hazard and/or exposure of the modified mosquito release product so that no additional data need be generated. Data requirements address both components of a risk assessment, i.e., the potential for hazard that the pesticide presents, and the estimated level of exposure to humans or nontarget species, including the potential for gene flow and dispersal.

## B. Regulatory History

*History of OX5034 Ae. aegypti males*

EPA issued an EUP in 2020 for the release of OX5034 *Ae. aegypti* mosquitoes. These releases were approved for limited releases of OX5034 mosquitoes in Monroe, Co., Florida and Harris, Co., Texas over two years across 6,600 acres. EPA concluded that no adverse effects are anticipated for any nontarget organisms, including endangered and threatened species,[1] as a result of the experimental permit to release OX5034 *Ae. aegypti* mosquitoes. That conclusion was based on literature reviews, species-specific behavioral traits of *Ae. aegypti*, the generalist nature of predators that consume mosquitoes, bioinformatics analyses, and toxicity studies [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>558</RecNum><Display Text>(USEPA 2020b)</DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629394477">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti; Data and Information Were Provided in Support of a FIFRA Section 5 Application. Memo from Wiebke Striegel and Amanda Pierce to Eric Bohnenblust, dated April 30,

[1] As to threatened and endangered species, since EPA concluded that there is a reasonable expectation of no discernible effects for nontarget organisms as a result of the experimental use permit to release OX5034 *Ae. aegypti* male mosquitoes, a "No Effect" determination was also made for direct and indirect effects to federally listed endangered and threatened species, and for their designated critical habitats.



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2020.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. A different transgenic mosquito developed by Oxitec, OX513A, is not covered under the current EUP.

*Related EPA approved products*

Since 2016, the pesticidal efficacy of WB1 *Ae. aegypti* males, a *Wolbachia pipientis* microbial pesticide for the suppression of localized *Ae. aegypti* mosquito populations, has been investigated under EPA approved EUPs. EPA previously concluded that the experimental work approved for 89669-EUP-3 presented negligible risks to humans, nontarget organisms, and the environment [ ADDIN EN.CITE ADDIN EN.CITE.DATA ]. The previous risk assessments concluded that the human health risks are negligible because exposure is negligible and that no adverse effects are expected for nontarget organisms because no hazard from exposure is expected if exposure to the *Wolbachia* pesticide occurs. Although the active ingredient for OX5034 *Ae. aegypti* mosquitoes and WB1 *Ae. aegypti* mosquitoes differs, considerations relating to *Ae. aegypti* biology and habitat, and the consideration of indirect effects to nontarget organisms due to the reduction of *Ae. aegypti*, are similar between the two products.

In 2017, EPA approved the product ZAP Males for a FIFRA Section 3 registration to manufacture and sell an end-use product consisting of male *Ae. albopictus* mosquitoes infected with the ZAP strain of *W. pipientis*. EPA evaluated direct and indirect effects to nontarget organisms from ZAP Males and concluded that no adverse effects were anticipated for nontarget organisms as a result of the registration for ZAP Males due to the same rationale as described for WB1 males [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2017</Year><RecNum>563</RecNum><Display Text>(USEPA 2017b)</DisplayText><record><rec-number>563</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629394762">563</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Revised Ecological Risk Assessment for the Section 3 registration of the microbial pesticide end-use product ZAP mosquito larvae: PC Code: 069035; EPA File Symbol 89668-U; Decision No. 513757; Submission Nos. 980717, 985153; DP Barcode No: 432413, 433706. MRID Nos: 49530604, 49830704-06. Memo from Shannon Borges to Wiebke Tapken, dated June 06, 2017.</title></titles><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>]. Although the active ingredient and mosquito species differ between ZAP Males and OX5034 males, many of the risk assessment considerations pertaining to potential exposure and indirect effects are similar between the two products.

### C. Mode of Action

OX5034 is described as a species-specific female-lethal trait that results in emergence of all-male progeny in the absence of tetracycline in the larval diet. The pesticidal effect of OX5034 is species-specific as it only affects the reproductive success of *Ae. aegypti* through mating between OX5034 *Ae. aegypti* males and *Ae. aegypti* females that are already present in the release area. OX5034 homozygous males alone will be released into the environment. Only female offspring from OX5034 matings are killed, while OX5034 hemizygous males survive to pass on the OX5034 female-lethal trait through further matings with wild type female *Ae. aegypti* mosquitoes. Unlike female mosquitoes, male mosquitoes do not bite humans. With continued field releases of OX5034 homozygous males, the *Ae. aegypti* population in the treatment area is thought to progressively decline due to the reduced number of females emerging each consecutive generation. In addition, OX5034 also expresses DsRed2-OX5034, a variant of the DsRed fluorescent protein form *Discosoma* spp., that allows for the visual identification of OX5034 hemizygous larvae collected from the field.

## III. Environmental Effects Assessment

### 1. Submitted environmental data

**Commented [WBJ17]:** The outline format changes, here, from "I.A.," etc. to "III.1.a," etc. It should be uniform, and I think it should be the former.

The applicant submitted laboratory toxicity studies and scientific rationale to fulfill non-target organism data requirements under the previously approved EUP [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>558</RecNum><DisplayText>(USEPA 2020b)</DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629394477">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti; Data and Information Were Provided in Support of a FIFRA Section 5 Application. Memo from Wiebke Striegel and Amanda Pierce to Eric Bohnenblust, dated April 30, 2020.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. No new studies for data requirements were required or submitted for the extension and amendment of the EUP, but the applicant did provide an updated literature review and scientific rationale pertaining to threatened and endangered species in the newly proposed testing locations. A summary of this is provided in Table 1. Some of the references cited in this assessment were included in rationale provided by the applicant within the MRID cited. Other references were included from the previous risk assessment and the open literature that pertained to specific topics discussed below.

**Table 1.** MRID submitted for the amendment and extension of the EUP.

| Data Requirement | OPPTS Guideline No. | Results Summary and Classification | MRID No. |
|---|---|---|---|
| Endangered Species Assessment | N/A | A literature review was provided as an analysis of the potential impact of OX5034 *Aedes aegypti* on threatened and endangered species or critical habitat in twelve counties in California and one county in Nevada. | 51361701 |

### 2. Nontarget organism exposure

Two postulated routes of exposure for nontarget organisms from the release of OX5034 *Ae. aegypti* male mosquitoes are dermal exposure and oral exposure. The two transgenic proteins produced in OX5034 *Ae. aegypti* male mosquitoes, DsRed2-OX5034 and tTAV-OX5034, are expressed in OX5034 tissues within the confines of its chitinaceous exoskeleton [ ADDIN EN.CITE

<EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>558</RecNum><DisplayText>(USEPA 2020b)</DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629394477">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti; Data and Information Were Provided in Support of a FIFRA Section 5 Application. Memo from Wiebke Striegel and Amanda Pierce to Eric Bohnenblust, dated April 30, 2020.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Therefore, both proteins are unavailable to nontarget organisms dermally should OX5034 males merely land on the surface of a nontarget organism. Female mosquitoes (non-species specific) take blood meals from humans and other animals, but because biting females will not be released under the requested EUP and male mosquitoes do not bite animals, nontarget organisms including listed species will not serve as bloodmeals for mosquitoes carrying tTAV-OX5034 and DsRed2-OX5034 proteins.

As described in EPA's 2020 risk assessment for the current OX5034 EUP, OX5034 female lethality is attributed to the overexpression of the tTAV-OX5034 protein in immature females, a process that is thought to interfere with the transcriptional machinery of the insect and consequently normal cellular function. As a result, females carrying the OX5034 trait survive only until the early larval stages unless tetracycline, which acts as a dietary antidote, is present at high enough levels. EPA confirmed via results from studies using mosquitoes from laboratory colonies and from field collections, that the OX5034 phenotype is 100% penetrant and that all females containing a copy of the OX5034 trait die prior to adulthood when reared in the absence of a tetracycline analogue [ ADDIN EN.CITE <EndNote><Cite ExcludeYear="1"><Author>MRID50889417</Author><Year>2019</Year><RecNum>568</RecNum><DisplayText>(MRID50889417 ; MRID50889423 ; MRID50889428)</DisplayText><record><rec-number>568</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629395463">568</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50889417,</author></authors></contributors><titles><title>The self-limiting phenotype, penetrance, longevity and egg clutch size of Aedes aegypti, OX5034</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite><Cite ExcludeYear="1"><Author>MRID50889423</Author><Year>2019</Year><RecNum>569</RecNum><record><rec-number>569</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629395488">569</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50889423,</author></authors></contributors><titles><title>Evaluation of field penetrance of OX5034 in open release field trials in Indaiatuba, Sao Paolo State, Brazil</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite><Cite ExcludeYear="1"><Author>MRID50889428</Author><Year>2019</Year><RecNum>570</RecNum><record><rec-number>570</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629395555">570</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50889428</author></authors></contributors><titles><title>Supplemental information in support of the study, evaluation of field penetrance of OX5034 in open release field trials in Indaiatuba, Sao Paolo State, Brazil</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]. Because the presence of tetracycline(s) in the environment may affect survivability of female OX5034 mosquitoes, the likelihood that OX5034 mosquitoes would encounter tetracycline sources at levels high enough for rescue from the lethal phenotype was previously evaluated [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>558</RecNum><DisplayText>(USEPA 2020b)</DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629394477">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti; Data and Information Were Provided in Support of a FIFRA Section 5 Application. Memo from Wiebke Striegel and Amanda Pierce to Eric Bohnenblust, dated April 30, 2020.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Several lines of evidence including a survey of environmental levels of tetracycline, tetracycline dose-response testing of OX5034 females, and oviposition behavior of *Ae. aegypti*, indicate that the risk of hemizygous OX5034 female mosquitoes emerging in the environment due to high levels of tetracycline is low [ ADDIN EN.CITE <EndNote><Cite ExcludeYear="1"><Author>MRID50889415</Author><Year>2019</Year><RecNum>571</RecNum><DisplayText>(MRID50889415)</DisplayText><record><rec-number>571</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629395715">571</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50889415,</author></authors></contributors><titles><title>Dose Response of Hemizygous Aedes aegypti OX5034 to Tetracyclines and Effects of Environmental Exposure to Tetracyclines</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]. A term of the original EUP restricts releases from occurring within 500 meters of potential tetracycline sources (i.e., sewage treatment facilities and any farms producing citrus crops) [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>572</RecNum><DisplayText>(USEPA 2020a)</DisplayText><record><rec-number>572</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629395808">572</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA</author></authors></contributors><titles><title>Experimental Use Permit Issued for 93167-EUP-2 to Allow for Releases of OX5034 Aedes aegypti in Florida and Texas</title></titles><dates><year>2020</year></dates><urls><related-urls><url>available at https://beta.regulations.gov/document/EPA-HQ-OPP-2019-0274-0353</url></related-urls></urls></record></Cite></EndNote>], and this term would continue under the EUP extension and amendment. The restriction in release locations resulted in the determination that the exposure to female mosquitoes is negligible and the exclusion of female biting as a dermal exposure pathway to the tTAV-OX5034 and DsRed2-OX5034 proteins [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>558</RecNum><DisplayText>(USEPA 2020b)</DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5"

timestamp="1629394477">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti; Data and Information Were Provided in Support of a FIFRA Section 5 Application. Memo from Wiebke Striegel and Amanda Pierce to Eric Bohnenblust, dated April 30, 2020.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

**Commented [PA18]:** Flagging to update based on the DER evaluating CA sites once that has been completed.

Regarding possible oral exposure, insect-eating animals, by definition, eat insects, and as mosquitoes are an insect it is possible for mosquitoes to be consumed by insect-eating animals. Given that it is possible that insect-eating animals might consume OX5034 *Ae. aegypti* male mosquitoes, EPA performed a comprehensive evaluation of the likely routes of exposure to OX5034 *Ae. aegypti* male mosquitoes for nontarget organisms, which includes listed species, as part of its 2020 risk assessment as summarized below.

*Ae. aegypti* is a major disease vector for humans, as female *Ae. aegypti* mosquitoes are known to vector diseases such as yellow fever, Zika, chikungunya, and dengue [ ADDIN EN.CITE <EndNote><Cite><Author>Nelson</Author><Year>1986</Year><RecNum>69</RecNum><DisplayText>(Christophers 1960; Nelson 1986)</DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507922481">69</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Nelson, M.J.</author></authors><secondary-authors><author>Organiztaion PAH</author></secondary-authors></contributors><titles><title><style face="italic" font="default" size="100%">Aedes aegypti</style><style face="normal" font="default" size="100%">: Biology and Ecology</style></title></titles><dates><year>1986</year></dates><pub-location>Washington, D.C.</pub-location><urls></urls></record></Cite><Cite><Author>Christophers</Author><Year>1960</Year><RecNum>573</RecNum><record><rec-number>573</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629396032">573</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Christophers, R.</author></authors></contributors><titles><title>Aedes aegypti (L.) The Yellow Fever Mosquito: Its Life History, Bionomics and Structure</title></titles><dates><year>1960</year></dates><publisher>Cambridge University Press</publisher><urls></urls></record></Cite></EndNote>]. The same dietary and habitat preferences that make *Ae. aegypti* females a deadly vector to humans are what limit the exposure of nontarget organisms to OX5034 *Ae. aegypti* male mosquitoes. Female *Ae. aegypti* preferentially feed on humans [ ADDIN EN.CITE <EndNote><Cite><Author>Harrington</Author><Year>2001</Year><RecNum>477</RecNum><DisplayText>(Harrington et al. 2001)</DisplayText><record><rec-number>477</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1578060419">477</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Harrington, L. C.</author><author>Edman, J. D.</author><author>Scott, T. W.</author></authors></contributors><titles><title>Why do female Aedes aegypti (Diptera : Culicidae) feed preferentially and frequently on human blood?</title><secondary-title>Journal of Medical Entomology</secondary-title></titles><periodical><full-title>Journal of Medical Entomology</full-

title></periodical><pages>411-422</pages><volume>38</volume><number>3</number><dates><year>2001</year><pub-dates><date>May</date></pub-dates></dates><isbn>0022-2585</isbn><accession-num>WOS:000168588900011</accession-num><urls><related-urls><url><Go to ISI>://WOS:000168588900011</url></related-urls></urls><electronic-resource-num>10.1603/0022-2585-38.3.411</electronic-resource-num></record></Cite></EndNote>] and therefore prefer that their breeding locations be near human dwellings in order to be in close proximity to their preferred food source [ ADDIN EN.CITE <EndNote><Cite><Author>Nelson</Author><Year>1986</Year><RecNum>69</RecNum><DisplayText>(Nelson 1986)</DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507922481">69</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Nelson, M.J.</author></authors><secondary-authors><author>Organiztaion PAH</author></secondary-authors></contributors><titles><title><style face="italic" font="default" size="100%">Aedes aegypti</style><style face="normal" font="default" size="100%">: Biology and Ecology</style></title></titles><dates><year>1986</year></dates><pub-location>Washington, D.C.</pub-location><urls></urls></record></Cite></EndNote>]. As such, *Ae. aegypti* usually uses man-made containers such as gutters, water containers, cans, and tires as breeding sites. Larval and pupal development occur in these breeding containers, completing the life cycle with adult emergence. Although *Ae. aegypti* historically bred in tree holes and other phytotelmata in sub-Saharan Africa prior to its introduction to the Americas [ ADDIN EN.CITE <EndNote><Cite><Author>Nelson</Author><Year>1986</Year><RecNum>69</RecNum><DisplayText>(Nelson 1986; Powell and Tabachnick 2013)</DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507922481">69</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Nelson, M.J.</author></authors><secondary-authors><author>Organiztaion PAH</author></secondary-authors></contributors><titles><title><style face="italic" font="default" size="100%">Aedes aegypti</style><style face="normal" font="default" size="100%">: Biology and Ecology</style></title></titles><dates><year>1986</year></dates><pub-location>Washington, D.C.</pub-location><urls></urls></record></Cite><Cite><Author>Powell</Author><Year>2013</Year><RecNum>67</RecNum><record><rec-number>67</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507919411">67</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Powell, J. R.</author><author>Tabachnick, W. J.</author></authors></contributors><titles><title>History of domestication and spread of Aedes aegypti - A Review</title><secondary-title>Memorias Do Instituto Oswaldo Cruz</secondary-title></titles><periodical><full-title>Memorias Do Instituto Oswaldo Cruz</full-title></periodical><pages>11-17</pages><volume>108</volume><dates><year>2013</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>0074-0276</isbn><accession-num>WOS:000330037800003</accession-num><urls><related-urls><url><Go to ISI>://WOS:000330037800003</url></related-urls></urls><electronic-resource-num>10.1590/0074-0276130395</electronic-resource-num></record></Cite></EndNote>], it is now well adapted to humans, flourishes in urban areas, and typically breeds in a number of artificial containers. It is possible for *Ae. aegypti* in the United States to also use tree holes or rock holes as breeding sites, but due to *Ae. aegypti's* high affinity for humans in the Americas [ ADDIN EN.CITE <EndNote><Cite><Author>Powell</Author><Year>2013</Year><RecNum>67</RecNum><DisplayTe

xt>(Powell and Tabachnick 2013)</DisplayText><record><rec-number>67</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507919411">67</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Powell, J. R.</author><author>Tabachnick, W. J.</author></authors></contributors><titles><title>History of domestication and spread of Aedes aegypti - A Review</title><secondary-title>Memorias Do Instituto Oswaldo Cruz</secondary-title></titles><periodical><full-title>Memorias Do Instituto Oswaldo Cruz</full-title></periodical><pages>11-17</pages><volume>108</volume><dates><year>2013</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>0074-0276</isbn><accession-num>WOS:000330037800003</accession-num><urls><related-urls><url><Go to ISI>://WOS:000330037800003</url></related-urls></urls><electronic-resource-num>10.1590/0074-0276130395</electronic-resource-num></record></Cite></EndNote>], *Ae. aegypti* is rarely found more than 100 meters from human dwellings [ ADDIN EN.CITE <EndNote><Cite><Author>Nelson</Author><Year>1986</Year><RecNum>69</RecNum><DisplayText>(Nelson 1986)</DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507922481">69</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Nelson, M.J.</author></authors><secondary-authors><author>Organiztaion PAH</author></secondary-authors></contributors><titles><title><style face="italic" font="default" size="100%">Aedes aegypti</style><style face="normal" font="default" size="100%">: Biology and Ecology</style></title></titles><dates><year>1986</year></dates><pub-location>Washington, D.C.</pub-location><urls></urls></record></Cite></EndNote>]. This proximity to human dwellings further reduces the likelihood of OX5034 *Ae. aegypti* larvae encountering nontarget organisms even in instances where the OX5034 larvae are found in more natural habitats such as tree holes or rock pools.

It is relevant to note that the [ ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Hribar</Author><Year>2001</Year><RecNum>504</RecNum><DisplayText>Hribar et al. (2001)</DisplayText><record><rec-number>504</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1584551797">504</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hribar, L. J.</author><author>Smith, J. M.</author><author>Vlach, J. J.</author><author>Verna, T. N.</author></authors></contributors><titles><title>Survey of container-breeding mosquitoes from the Florida Keys, Monroe County, Florida</title><secondary-title>Journal of the American Mosquito Control Association</secondary-title></titles><periodical><full-title>Journal of the American Mosquito Control Association</full-title></periodical><pages>245-248</pages><volume>17</volume><number>4</number><dates><year>2001</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>8756-971X</isbn><accession-num>WOS:000175646800007</accession-num><urls><related-urls><url><Go to ISI>://WOS:000175646800007</url></related-urls></urls></record></Cite></EndNote>] and [ ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Hribar</Author><Year>2004</Year><RecNum>502</RecNum><DisplayText>Hribar et al. (2004)</DisplayText><record><rec-number>502</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1584548144">502</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hribar, L. J.</author><author>Vlach, J. J.</author><author>DeMay, D. J.</author><author>James, S. S.</author><author>Fahey, J.

S.</author><author>Fussell, E. M.</author></authors></contributors><titles><title>Mosquito larvae (Culicidae) and other Diptera associated with containers, storm drains, and sewage treatment plants in the Florida Keys, Monroe County, Florida</title><secondary-title>Florida Entomologist</secondary-title></titles><periodical><full-title>Florida Entomologist</full-title></periodical><pages>199-203</pages><volume>87</volume><number>2</number><dates><year>2004</year><pub-dates><date>Jun</date></pub-dates></dates><isbn>0015-4040</isbn><accession-num>WOS:000222319500016</accession-num><urls><related-urls><url><Go to ISI>://WOS:000222319500016</url></related-urls></urls><electronic-resource-num>10.1653/0015-4040(2004)087[0199:mlcaod]2.0.co;2</electronic-resource-num></record></Cite></EndNote>] studies referenced in EPA's 2020 risk assessment are mosquito habitat surveys specific to one of the EUP locations (Monroe County, FL). Therefore, findings such as "[o]n Big Pine Key and Vaca Key, mosquito larvae were most often collected from tires, whereas on Key West most collections were made from flowerpots, planters, and trivets" described in [ ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Hribar</Author><Year>2004</Year><RecNum>502</RecNum><DisplayText>Hribar et al. (2004)</DisplayText><record><rec-number>502</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1584548144">502</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hribar, L. J.</author><author>Vlach, J. J.</author><author>DeMay, D. J.</author><author>James, S. S.</author><author>Fahey, J. S.</author><author>Fussell, E. M.</author></authors></contributors><titles><title>Mosquito larvae (Culicidae) and other Diptera associated with containers, storm drains, and sewage treatment plants in the Florida Keys, Monroe County, Florida</title><secondary-title>Florida Entomologist</secondary-title></titles><periodical><full-title>Florida Entomologist</full-title></periodical><pages>199-203</pages><volume>87</volume><number>2</number><dates><year>2004</year><pub-dates><date>Jun</date></pub-dates></dates><isbn>0015-4040</isbn><accession-num>WOS:000222319500016</accession-num><urls><related-urls><url><Go to ISI>://WOS:000222319500016</url></related-urls></urls><electronic-resource-num>10.1653/0015-4040(2004)087[0199:mlcaod]2.0.co;2</electronic-resource-num></record></Cite></EndNote>] provide additional certainty that habitat preferences of *Ae. aegypti* described in the general scientific literature also hold true at the EUP locations. Of relevance to the current action, in Stanislaus and Los Angeles Counties in California, *Aedes aegypti* breeding sources were identified in improperly sealed rain barrels, drains, pots, old cars, and filters of unused hot tubs [ ADDIN EN.CITE ADDIN EN.CITE.DATA ]. These findings again provide additional certainty that habitat preferences of *Ae. aegypti* described in the general scientific literature hold true at the newly proposed EUP locations in California as well. The use of these man-made containers as larval habitat and breeding sites greatly reduces the likelihood of nontarget organisms, which includes listed species, encountering OX5034 *Ae. aegypti* larvae.

The anthropophilic nature of *Ae. aegypti* mosquitoes also reduces the likelihood of nontarget organisms encountering OX5034 *Ae. aegypti* adult males. This is because *Ae. aegypti* adults are typically found near or even inside human dwellings, thus limiting their availability to predators [ ADDIN EN.CITE <EndNote><Cite><Author>Christophers</Author><Year>1960</Year><RecNum>573</RecNum><DisplayText>(Christophers 1960)</DisplayText><record><rec-number>573</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629396032">573</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Christophers, R.</author></authors></contributors><titles><title>Aedes aegypti (L.) The Yellow Fever Mosquito: Its Life History, Bionomics and

Structure</title></titles><dates><year>1960</year></dates><publisher>Cambridge University Press</publisher><urls></urls></record></Cite></EndNote>]. Adult *Ae. aegypti* mosquitoes typically rest on walls and in shaded areas within and around human dwellings [ ADDIN EN.CITE ADDIN EN.CITE.DATA ]. As previously discussed, *Ae. aegypti* are adapted to domestic and urban environments that allow females easy and unlimited access to blood meals, such as those around human habitations. Although only females take blood meals, *Ae. aegypti* males also frequent human habitations in order to maintain proximity to females for mating [ ADDIN EN.CITE <EndNote><Cite><Author>Nelson</Author><Year>1986</Year><RecNum>69</RecNum><DisplayText>(Nelson 1986)</DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507922481">69</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Nelson, M.J.</author></authors><secondary-authors><author>Organiztaion PAH</author></secondary-authors></contributors><titles><title><style face="italic" font="default" size="100%">Aedes aegypti</style><style face="normal" font="default" size="100%">: Biology and Ecology</style></title></titles><dates><year>1986</year></dates><pub-location>Washington, D.C.</pub-location><urls></urls></record></Cite></EndNote>]. Due to the anthropophilic nature of the target pest, OX5034 *Ae. aegypti* releases occur in residential sites. As *Ae. aegypti* dispersal is generally limited to around 200 meters based on worldwide release recapture studies [ ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>511</RecNum><DisplayText>(OECD 2018)</DisplayText><record><rec-number>511</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1585148502">511</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Safety Assessment of Transgenic Organisms in the Environment, Volume 8</title></titles><dates><year>2018</year></dates><urls><related-urls><url>https://www.oecd-ilibrary.org/content/publication/9789264302235-en</url></related-urls></urls><electronic-resource-num>doi:https://doi.org/10.1787/9789264302235-en</electronic-resource-num></record></Cite></EndNote>], released OX5034 *Ae. aegypti* will not travel far from the release site. This proximity to residential sites again limits the nontarget organisms that may encounter OX5034 *Ae. aegypti* male mosquitoes.

Therefore, due to species-specific behavioral traits of *Ae. aegypti* resulting in its preferential habitat being largely limited to areas surrounding human dwellings and its preferential breeding sites being largely composed of man-made containers, the potential of exposure of nontarget organisms to OX5034 *Ae. aegypti* males is limited. Due to the OX5034 *Ae. aegypti* releases occurring in residential sites and to biological traits of *Ae. aegypti* (e.g., anthropophilic, limited dispersal), it is reasonable to find that exposure to OX5034 *Ae. aegypti* mosquitoes by listed species is expected to be limited.

**3. Nontarget effects**

**a. Direct effects**

No new studies were submitted for the extension and amendment of the EUP, but EPA previously evaluated whether there was any risk to nontarget organisms, which includes listed species, from the consumption of the OX5034 *Ae. aegypti* male mosquito, and EPA found that no adverse effects from consumption are expected – that is, there is a reasonable expectation of no discernible effects for nontarget organisms – based on the mode of toxicity of tTAV, bioinformatics analyses, and acute oral toxicity studies [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>558</RecNum><DisplayText>(USEPA 2020b)</DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629394477">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti; Data and Information Were Provided in Support of a FIFRA Section 5 Application. Memo from Wiebke Striegel and Amanda Pierce to Eric Bohnenblust, dated April 30, 2020.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. As described in EPA's 2020 risk assessment, the pesticidal effect of OX5034 is species-specific as it only affects the reproductive success of *Ae. aegypti* through mating between OX5034 *Ae. aegypti* males and *Ae. aegypti* females that are already present in the release area. As OX5034 female lethality is due to the overproduction of tTAV-OX5034 protein inside of the female's own cells, this non-toxic mode of action ~~makes it unlikely~~renders a reasonable expectation that the OX5034 transgenic proteins would be non-toxic to non-target organisms – and thus that there would be no discernable effects – upon consumption of OX5034 *Ae. aegypti* male mosquitoes. Based upon bioinformatic analysis, neither the DsRed2-OX5034 or tTAV-OX5034 proteins share significant sequence similarity with known toxins [ ADDIN EN.CITE <EndNote><Cite ExcludeYear="1"><Author>MRID50889420</Author><Year>2019</Year><RecNum>574</RecNum><DisplayText>(MRID50889420)</DisplayText><record><rec-number>574</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629396474">574</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50889420,</author></authors></contributors><titles><title>Bioinformatics analysis for risks of allergenicity and toxicity of proteins encoded by the two genes introduced into genetically engineered mosquitoes (Aedes aegypti) strain OX5034.</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]. Both proteins are predicted to be susceptible to several proteases found in the human digestive system (i.e., pepsin, trypsin, chymotrypsin) based upon these bioinformatics analyses, and thus the proteins are expected to be broken down following ingestion.

In addition to bioinformatics analyses, EPA also evaluated toxicity studies which indicated that fish and freshwater invertebrates that ingest OX5034 *Ae. aegypti* male mosquitoes are not adversely affected [ ADDIN EN.CITE ADDIN EN.CITE.DATA ]. A submitted study tested the potential toxicity of OX5034 *Ae. aegypti* male mosquitoes fed to guppies to evaluate the direct impact of consumption of OX5034 mosquitoes on nontarget aquatic vertebrate organisms [ ADDIN EN.CITE <EndNote><Cite ExcludeYear="1"><Author>MRID50889408</Author><Year>2019</Year><RecNum>576</RecNum><DisplayText>(MRID50698708 ; MRID50889408)</DisplayText><record><rec-number>576</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629397064">576</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50889408,</author></authors></contributors><titles><title>Supplemental Information in Support of the Study, A laboratory toxicity study to determine the effects

**Commented [WBJ19]:** BPPD – please ensure that my suggested edit here (which is aimed at the ESA determination) is accurate.

**Commented [WBJ20]:** BPPD – please ensure that my suggested edits accurately reflect your meaning. I'm concerned that the use of the term "unlikely" could be read as a "may effect – not likely to adversely affect" ("NLAA") finding as to listed species, which would require informal consultation with the Services.

**Commented [WBJ21]:** But we're talking about other "non-targets" besides humans here, right?

of Aedes aegypti strain OX5034 towards Poecilia reticulata (Actinopterygii: Poeciliidae) under semi-static conditions (Syntech Study No 232SRRES18C01).</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite><Cite ExcludeYear="1"><Author>MRID50698708</Author><Year>2019</Year><RecNum>577</RecNum><record><rec-number>577</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629397103">577</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50698708,</author></authors></contributors><titles><title>A laboratory toxicity study to determine the effects of Aedes aegypti strain OX5034 towards Poecilia reticulata (Actinopterygii: Poeciliidae) under semi-static conditions.</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]. The study found no acute or sublethal adverse effects to the test organisms over the 14-day test period, indicating ~~that the likelihood for adverse~~ a reasonable expectation of no discernable effects to nontarget aquatic vertebrates from consumption of OX5034 *Ae. aegypti* mosquitoes ~~is low~~.

**Commented [WBJ22]:** BPPD – please ensure that my suggested edits accurately reflect your meaning. I'm concerned that the use of the term "likelihood … is low" could be read as a "may effect – not likely to adversely affect" ("NLAA") finding as to listed species, which would require informal consultation with the Services.

To evaluate the direct impact on nontarget aquatic invertebrate organisms through oral consumption of OX5034 *Ae. aegypti*, a submitted study tested the potential toxicity of OX5034 *Ae. aegypti* male mosquitoes to an aquatic invertebrate. A feeding study examined the American signal crayfish [ ADDIN EN.CITE <EndNote><Cite ExcludeYear="1"><Author>MRID50889407</Author><Year>2019</Year><RecNum>575</RecNum><DisplayText>(MRID50698707 ; MRID50889407)</DisplayText><record><rec-number>575</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629397046">575</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50889407</author></authors></contributors><titles><title>Supplemental Information in Support of the Study, Aedes aegypti strain OX5034 larvae (batch RD021018): 96 Hour Feeding Study with the American (Signal) Crayfish (Envigo Study No. VH34HP; EPA MRID 50698707).</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite><Cite ExcludeYear="1"><Author>MRID50698707</Author><Year>2019</Year><RecNum>578</RecNum><record><rec-number>578</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629397118">578</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50698707,</author></authors></contributors><titles><title>Aedes aegypti strain OX5034 larvae (batch RD021018): 96 Hour Feeding Study with the American (Signal) Crayfish.</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>] and found no acute or sublethal adverse effects to the test organisms when fed OX5034 mosquitoes over a 96-hour test period. As crayfish are larger in mass than juvenile aquatic insects and may therefore be less sensitive to low level toxins, EPA recommended an aquatic insect larval study be performed prior to a Section 3 registration for additional certainty regarding transferability of the study conclusions to juvenile aquatic insects [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>558</RecNum><DisplayText>(USEPA 2020b)</DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629394477">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Huma n Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti; Data and Information Were Provided in Support of a FIFRA Section 5 Application. Memo from Wiebke Striegel and Amanda Pierce to Eric Bohnenblust, dated April 30, 2020.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Although the use of aquatic insect larva rather than crayfish as a test organism for an aquatic invertebrate study was recommended, it should be noted that in the evaluation of the waiver for nontarget insect testing, which was deemed acceptable, EPA stated that "concerns regarding oral consumption of OX5034 mosquitoes by insect species is not considered as a significant risk due to a lack of plausible toxicity to these species via uptake during normal digestive processes." [ ADDIN EN.CITE <EndNote><Cite><Author>USEPA</Author><Year>2020</Year><RecNum>579</RecNum><DisplayText>(USEPA 2020d)</DisplayText><record><rec-number>579</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629397230">579</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>USEPA,</author></authors></contributors><titles><title>Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034) and Related Genetic Material from OX5034 Aedes aegypti: Request for Waiver from Nontarget Insect Testing MRID: 50889413.</title></titles><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Of importance to the currently approved EUP, there are no listed (i.e., threatened or endangered) insect species in Monroe County, Florida with an aquatic larval stage (Table A1, Appendix). Regarding listed species in the newly requested EUP locations in California, no listed insect species in the requested counties have an aquatic larval stage, and other listed aquatic invertebrates are crustaceans, which would not be expected to be adversely affected should they consume OX5034 *Ae. aegypti* larvae due to the submitted aquatic invertebrate toxicity study in crayfish (Table A2, Appendix).

**Commented [WBJ23]:** If this is the end of the quoted sentence, then this should be a period and not a comma.

In summary, no adverse effects to nontarget organisms at the taxa level, which necessarily includes listed species, are expected from the consumption of OX5034 *Ae. aegypti* male mosquitoes based on 1) bioinformatics analyses demonstrating lack of similarity between DsRed2-OX5034 or tTAV-OX5034 and known toxins, 2) bioinformatics analyses demonstrating susceptibility of DsRed2-OX5034 or tTAV-OX5034 to gastric proteases, 3) toxicity study indicating no adverse effects to fish upon OX5034 *Ae. aegypti* male mosquito consumption, and 4) toxicity study indicating no adverse effects to an aquatic invertebrate upon OX5034 *Ae. aegypti* male mosquito consumption.

**Commented [WBJ24]:** Can we say "discernable," here, for ESA reasons?

**b. Indirect effects**

As described in EPA's 2020 risk assessment, based on a review of the scientific literature, no published papers have been located that identify any predator species that is dependent on *Ae. aegypti* as a crucial component of its diet [ ADDIN EN.CITE ADDIN EN.CITE.DATA ]. There are over 3,000 species of mosquitoes worldwide with approximately 176 species of mosquitoes in the United States, and *Ae. aegypti* is but one. For example, *Ae. aegypti* make up less than 2% of the mosquito species caught in traps in Monroe County, Florida [ ADDIN EN.CITE <EndNote><Cite ExcludeYear="1"><Author>MRID50889414</Author><Year>2019</Year><RecNum>580</RecNum><DisplayText>(MRID50889414)</DisplayText><record><rec-number>580</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5"

timestamp="1629397611">580</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>MRID50889414,</author></authors></contributors><titles><title>Analysis of no effect to threatened or endangered species or critical habitat</title></titles><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>], making it unlikelyrendering a reasonable expectation that *Ae. aegypti* would do not play a critical role in the diet of any predators, including listed species. Nonetheless, in order to comprehensively evaluate the potential risk to nontarget organisms, including listed species, from the release of OX5034 *Ae. aegypti* male mosquitoes, as part of its 2020 risk assessment, EPA evaluated the potential for ecosystem level effects due to the possibility of population level reduction of the target pest. Organisms that consume, or are capable of consuming, mosquitoes (non-species specific) are typically considered to be dietary generalists largely due to an individual mosquito containing little caloric energy [ ADDIN EN.CITE <EndNote><Cite><Author>Wetzler</Author><Year>2018</Year><RecNum>499</RecNum><DisplayText>(Wetzler and Boyles 2018)</DisplayText><record><rec-number>499</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1584548144">499</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wetzler, G. C.</author><author>Boyles, J. G.</author></authors></contributors><titles><title>The energetics of mosquito feeding by insectivorous bats</title><secondary-title>Canadian Journal of Zoology</secondary-title></titles><periodical><full-title>Canadian Journal of Zoology</full-title></periodical><pages>373-377</pages><volume>96</volume><number>4</number><dates><year>2018</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>0008-4301</isbn><accession-num>WOS:000429352400011</accession-num><urls><related-urls><url><Go to ISI>://WOS:000429352400011</url></related-urls></urls><electronic-resource-num>10.1139/cjz-2017-0162</electronic-resource-num></record></Cite></EndNote>], thereby making a dietary strategy that specializes on mosquitoes to be energetically costly. An example of this is a report of bat diet preferences in Florida that indicates that although the southeastern brown bats in Florida do ingest mosquitoes, they display a strong preference for beetles and moths in their diet [ ADDIN EN.CITE <EndNote><Cite><Author>Zinn</Author><Year>1981</Year><RecNum>507</RecNum><DisplayText>(Zinn and Humphrey 1981)</DisplayText><record><rec-number>507</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1584555190">507</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zinn, T.L.</author><author>Humphrey, S.R.</author></authors></contributors><titles><title><style face="normal" font="default" size="100%">Seasonal Food Resources and Prey Selection of the Southeastern Brown Bat </style><style face="italic" font="default" size="100%">Myotis austroriparius</style><style face="normal" font="default" size="100%"> in Florida USA</style></title><secondary-title>Florida Scientist</secondary-title></titles><periodical><full-title>Florida Scientist</full-title></periodical><pages>81-90</pages><volume>44</volume><dates><year>1981</year></dates><urls></urls><electronic-resource-num>10.2307/24319689</electronic-resource-num></record></Cite></EndNote>], which tend to be larger prey items and thus more energetically efficient targets. Likewise, the American Mosquito Control Association (AMCA) also reviewed the role of bats for mosquito control on its website, indicating that although bats do eat mosquitoes (non-species specific), mosquitoes comprised less than 1% of the gut contents of wild caught bats in the studies reviewed to date, and that other insects, such as moths provide better nutritional value [ ADDIN EN.CITE <EndNote><Cite Hidden="1"><Author>American Mosquito Control Association (AMCA)</Author><RecNum>582</RecNum><record><rec-number>582</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629418983">582</key></foreign-keys><ref-type name="Web Page">12</ref-type><contributors><authors><author>American Mosquito Control Association (AMCA),</author></authors></contributors><titles><title>Frequently asked

**Commented [WBJ25]:** BPPD – please ensure that my suggested edits accurately reflect your meaning. I'm concerned that the use of the term "unlikely" could be read as a "may effect – not likely to adversely affect" ("NLAA") finding as to listed species, which would require informal consultation with the Services.

questions</title></titles><volume>2021</volume><number>July 27, 2021</number><dates></dates><urls><related-urls><url>https://www.mosquito.org/page/faq</url></related-urls></urls></record></Cite></EndNote>](AMCA, 2021). Similarly, invertebrate predators like dragonflies are known to eat adult mosquitoes (non-species specific); however, they also consume butterflies, moths and smaller dragonflies, thus mosquitoes are likely not an essential part of their diet [ ADDIN EN.CITE <EndNote><Cite><Author>Pfitzner</Author><Year>2015</Year><RecNum>166</RecNum><DisplayText>(Pfitzner et al. 2015)</DisplayText><record><rec-number>166</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1508448111">166</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pfitzner, W. P.</author><author>Beck, M.</author><author>Weitzel, T.</author><author>Becker, N.</author></authors></contributors><titles><title>The role of mosquitoes in the diet of adult dragon and damselflies (Odonata)</title><secondary-title>Journal of the American Mosquito Control Association</secondary-title></titles><periodical><full-title>Journal of the American Mosquito Control Association</full-title></periodical><pages>187-189</pages><volume>31</volume><number>2</number><dates><year>2015</year><pub-dates><date>Jun</date></pub-dates></dates><isbn>8756-971X</isbn><accession-num>WOS:000357707000011</accession-num><urls><related-urls><url><Go to ISI>://WOS:000357707000011</url></related-urls></urls></record></Cite></EndNote>], and no dragonflies are known to rely on *Ae. aegypti* as a food source.

It is also pertinent to note *Ae. aegypti* is an invasive species in the United States. Given its relatively recent arrival [ ADDIN EN.CITE <EndNote><Cite><Author>Powell</Author><Year>2013</Year><RecNum>67</RecNum><DisplayText>(Powell and Tabachnick 2013)</DisplayText><record><rec-number>67</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507919411">67</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Powell, J. R.</author><author>Tabachnick, W. J.</author></authors></contributors><titles><title>History of domestication and spread of Aedes aegypti - A Review</title><secondary-title>Memorias Do Instituto Oswaldo Cruz</secondary-title></titles><periodical><full-title>Memorias Do Instituto Oswaldo Cruz</full-title></periodical><pages>11-17</pages><volume>108</volume><dates><year>2013</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>0074-0276</isbn><accession-num>WOS:000330037800003</accession-num><urls><related-urls><url><Go to ISI>://WOS:000330037800003</url></related-urls></urls><electronic-resource-num>10.1590/0074-0276130395</electronic-resource-num></record></Cite></EndNote>], *Ae. aegypti* is therefore unlikely to represent a keystone species or to have co-evolved any significant relationships with nontarget organisms in the United States. The expected lack of significant relationships further supports the expectation that nontarget organisms do not specifically rely on *Ae. aegypti* for food. For the newly proposed EUP locations in particular, breeding populations of *Ae. aegypti* were only first discovered in California in 2013 [ ADDIN EN.CITE <EndNote><Cite><Author>Gloria-Soria</Author><Year>2014</Year><RecNum>585</RecNum><DisplayText>(Gloria-Soria et al. 2014)</DisplayText><record><rec-number>585</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629419934">585</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gloria-Soria, A.</author><author>Brown, J. E.</author><author>Kramer, V.</author><author>Yoshimizu, M. H.</author><author>Powell, J. R.</author></authors></contributors><titles><title>Origin of the Dengue

Fever Mosquito, Aedes aegypti, in California</title><secondary-title>Plos Neglected Tropical Diseases</secondary-title></titles><periodical><full-title>Plos Neglected Tropical Diseases</full-title></periodical><volume>8</volume><number>7</number><dates><year>2014</year><pub-dates><date>Jul</date></pub-dates></dates><isbn>1935-2735</isbn><accession-num>WOS:000340551500058</accession-num><urls><related-urls><url><Go to ISI>://WOS:000340551500058</url></related-urls></urls><custom7>e3029</custom7><electronic-resource-num>10.1371/journal.pntd.0003029</electronic-resource-num></record></Cite></EndNote>], making it extremely unlikely that any nontarget organism would have become dependent on *Ae. aegypti* as a food source within a decade.

The recent arrival of *Ae. aegypti* similarly results in the expectation that it is unlikely to play a critical role in other ecosystem functions, such as pollination. Although female mosquitoes take blood meals from humans, mosquitoes of both sexes require plant juices as an energy source. Floral nectars are the best-known sources, but mosquitoes (non-species specific) are also known to obtain sugars from extra- floral nectaries, damaged fruits, damaged and intact vegetative tissues, and honeydew [ ADDIN EN.CITE <EndNote><Cite><Author>Clements</Author><Year>2000</Year><RecNum>134</RecNum><DisplayText>(Clements 2000)</DisplayText><record><rec-number>134</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1508260882">134</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Clements, A.N.</author></authors></contributors><titles><title>Nutrition and reproduction</title><secondary-title>The biology of mosquitoes</secondary-title></titles><volume>1</volume><edition>2nd</edition><dates><year>2000</year></dates><pub-location>Oxford</pub-location><publisher>CABI Publishing</publisher><urls></urls></record></Cite></EndNote>]. As *Ae. aegypti* are adapted to domestic and urban environments that tend to be low in sugar sources, it is likely that *Ae. aegypti* males are reliant on sugar sources from potted plants or plant species that are found around houses [ ADDIN EN.CITE <EndNote><Cite><Author>Martinez-Ibarra</Author><Year>1997</Year><RecNum>143</RecNum><DisplayText>(Martinez-Ibarra et al. 1997)</DisplayText><record><rec-number>143</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1508428003">143</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Martinez-Ibarra, J. A.</author><author>Rodriguez, M. H.</author><author>Arredondo-Jimenez, J. I.</author><author>Yuval, B.</author></authors></contributors><titles><title>Influence of plant abundance on nectar feeding by Aedes aegypti (Diptera : Culicidae) in southern Mexico</title><secondary-title>Journal of Medical Entomology</secondary-title></titles><periodical><full-title>Journal of Medical Entomology</full-title></periodical><pages>589-593</pages><volume>34</volume><number>6</number><dates><year>1997</year><pub-dates><date>Nov</date></pub-dates></dates><isbn>0022-2585</isbn><accession-num>WOS:000071212100002</accession-num><urls><related-urls><url><Go to ISI>://WOS:000071212100002</url></related-urls></urls></record></Cite></EndNote>]. There are no reports that *Ae. aegypti* is an important pollinator for any plant species; this lack of pollination activity may be because, as a non-native species, *Ae. aegypti* has not been present in the ecosystem for sufficient time to develop an essential ecosystem function. Dedicated pollinator species for particular flowers typically require close evolution for many thousands of years [ ADDIN EN.CITE <EndNote><Cite><Author>Patiny</Author><Year>2012</Year><RecNum>142</RecNum><DisplayTe

**Commented [WBJ26]:** Again, the term “unlikely” is somewhat problematic. Since we’re talking about an “expectation,” can we say “the expectation that it does not play a critical role? Or maybe “reasonable expectation that it does not...?”

xt>(Patiny 2012)</DisplayText><record><rec-number>142</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1508427838">142</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Patiny, S.</author></authors></contributors><titles><title>Evolution of plant-pollinator relationships</title></titles><dates><year>2012</year></dates><pub-location>Cambridge</pub-location><publisher>Cambridge University Press</publisher><urls></urls></record></Cite></EndNote>].

Additionally, as *Ae. aegypti* is a major pest species with known impacts on human health by vectoring disease, it is continually suppressed by other control methods such as the use of chemical and microbial insecticides as well as breeding site source reduction [ ADDIN EN.CITE <EndNote><Cite><Author>Nelson</Author><Year>1986</Year><RecNum>69</RecNum><DisplayText>(Nelson 1986)</DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507922481">69</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Nelson, M.J.</author></authors><secondary-authors><author>Organiztaion PAH</author></secondary-authors></contributors><titles><title><style face="italic" font="default" size="100%">Aedes aegypti</style><style face="normal" font="default" size="100%">: Biology and Ecology</style></title></titles><dates><year>1986</year></dates><pub-location>Washington, D.C.</pub-location><urls></urls></record></Cite></EndNote>], which further reduces the likelihood that a predator would be dependent on *Ae. aegypti* as a food source or for other ecosystem functions.

Moreover, the species-specific behaviors of *Ae. aegypti* outlined as factors limiting exposure to nontarget organisms also limit the likelihood that predators would be reliant on this species. To this point, aquatic predator species tend to be rare or absent from man-made containers [ ADDIN EN.CITE ADDIN EN.CITE.DATA ]. Because *Ae. aegypti* usually uses man-made containers such as gutters, water containers, cans, and tires as breeding sites, there appears to be no specific predator that preys exclusively on *Ae. aegypti* in the aquatic stage, but, rather, predators that are generally opportunistic and feed on larvae or adults when they encounter them [ ADDIN EN.CITE <EndNote><Cite><Author>Christophers</Author><Year>1960</Year><RecNum>573</RecNum><DisplayText>(Christophers 1960)</DisplayText><record><rec-number>573</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1629396032">573</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Christophers, R.</author></authors></contributors><titles><title>Aedes aegypti (L.) The Yellow Fever Mosquito: Its Life History, Bionomics and Structure</title></titles><dates><year>1960</year></dates><publisher>Cambridge University Press</publisher><urls></urls></record></Cite></EndNote>]. This rationale also applies to adult *Ae. aegypti*, as they are typically found near or even inside human dwellings, thus providing some protection from predators [ ADDIN EN.CITE <EndNote><Cite><Author>Nelson</Author><Year>1986</Year><RecNum>69</RecNum><DisplayText>(Nelson 1986)</DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1507922481">69</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Nelson, M.J.</author></authors><secondary-authors><author>Organiztaion PAH</author></secondary-authors></contributors><titles><title><style face="italic" font="default" size="100%">Aedes aegypti</style><style face="normal" font="default" size="100%">: Biology and

Ecology</style></title></titles><dates><year>1986</year></dates><pub-location>Washington, D.C.</pub-location><urls></urls></record></Cite></EndNote>]. Moreover, as required by the anthropophilic nature of the target pest, OX5034 *Ae. aegypti* releases occur in residential sites. As *Ae. aegypti* dispersal is generally limited to around 200 meters based on worldwide release recapture studies [ ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>511</RecNum><DisplayText>(OECD 2018)</DisplayText><record><rec-number>511</rec-number><foreign-keys><key app="EN" db-id="9dafe52dcp9s9xe99drvp0fnwz0sz59xdwa5" timestamp="1585148502">511</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Safety Assessment of Transgenic Organisms in the Environment, Volume 8</title></titles><dates><year>2018</year></dates><urls><related-urls><url>https://www.oecd-ilibrary.org/content/publication/9789264302235-en</url></related-urls></urls><electronic-resource-num>doi:https://doi.org/10.1787/9789264302235-en</electronic-resource-num></record></Cite></EndNote>], released OX5034 *Ae. aegypti* will not travel far from the release site, therefore restricting access to predators.

In summary, no adverse effects to nontarget organisms at the taxa level, which necessarily includes listed species, are expected should OX5034 *Ae. aegypti* male mosquitoes successfully reduce the *Ae. aegypti* population in the EUP locations based on 1) literature reviews that indicate that no species are reliant on *Ae. aegypti* mosquitoes as a food source, 2) the generalist nature of predators that consume mosquitoes, 3) species-specific behavioral traits of *Ae. aegypti* that limit the potential for interaction with nontarget organisms, 4) the invasive species status of *Ae. aegypti* which reduces the likelihood that any significant co-evolutionary relationships exist with nontarget organisms in the United States, and 5) *Ae. aegypti* is commonly targeted for pest reduction through mosquito control measures which further limits the likelihood that a nontarget organism would be reliant upon this species for food.

**Commented [WBJ27]:** Can we say "discernable," here, for ESA reasons?

## IV. ENVIRONMENTAL RISK CONCLUSIONS

EPA considered possible routes of exposure to OX5034 *Ae. aegypti* male mosquitoes, the likelihood of a hazard from the consumption of OX5034 *Ae. aegypti* male mosquitoes, and the likelihood of a hazard from the possible reduction in the wild *Ae. aegypti* population leading to a possible reduction in a nontarget organism's food source. EPA then evaluated risk by examining the possible hazards and possible routes of exposure in conjunction (i.e., Risk = Hazard x Exposure). In events where exposure may be possible, but no hazard is identified, risk is concluded to be negligible.

EPA concluded that the potential of exposure of any nontarget organisms, which includes endangered and threatened species, to OX5034 *Ae. aegypti* male mosquitoes is limited due to species-specific behavioral traits of *Ae. aegypti* resulting in its preferential habitat being largely limited to areas surrounding human dwellings and its preferential breeding sites being largely composed of man-made containers.

EPA concluded that the consumption of OX5034 *Ae. aegypti* male mosquitoes by nontarget organisms is not expected to pose a hazard to any nontarget organisms, which includes endangered or threatened species based on 1) bioinformatics analyses demonstrating lack of similarity between DsRed2-OX5034 or tTAV-OX5034 and known toxins, 2) bioinformatics analyses demonstrating susceptibility of DsRed2-OX5034 or tTAV-OX5034 to gastric proteases, 3) toxicity study indicating no adverse effects to fish upon OX5034 *Ae. aegypti* male mosquito consumption, and 4) toxicity study indicating no adverse effects to an aquatic invertebrate upon OX5034 *Ae. aegypti* male mosquito consumption.

EPA concluded that the possible reduction of the *Ae. aegypti* populations in the EUP locations is not expected to pose a hazard to any nontarget organisms, which includes endangered or threatened species, based on 1) literature reviews that indicate that no species are reliant on *Ae. aegypti* mosquitoes as a food source, 2) the generalist nature of predators that consume mosquitoes, 3) species-specific behavioral traits of *Ae. aegypti* that limit the potential for interaction with nontarget organisms, 4) the invasive species status of *Ae. aegypti* which reduces the likelihood that any significant co-evolutionary relationships exist with nontarget organisms in the United States, and 5) *Ae. aegypti* is commonly targeted for pest reduction through mosquito control measures which further limits the likelihood that a nontarget organism would be reliant upon this species for food.

Therefore, although exposure may be possible (but is expected to be limited), and because no hazard was identified (i.e., no hazard from oral consumption or from the reduction of the local *Ae. aegypti* population), there is a reasonable expectation of no discernible effects for nontarget organisms as a result of the experimental use permit to release OX5034 *Ae. aegypti* male mosquitoes.

## V. RISK TO FEDERALLY LISTED THREATENED AND ENDANGERED SPECIES

EPA concluded there is a reasonable expectation of no discernible effects for nontarget organisms as a result of the experimental permit to release OX5034 *Ae. aegypti* male mosquitoes. Therefore, since no discernable effects are anticipated to any nontarget organism, a "No Effect" determination is also made for direct and indirect effects to federally listed endangered and threatened species, and for their designated critical habitats.

## VI. REFERENCES

[ ADDIN EN.REFLIST ]

## VI. APPENDIX

**Table A1.** Listed species in Monroe County, Florida sorted by Group then Scientific Name. Information from [ HYPERLINK "https://www.fws.gov/endangered/" ] last accessed 8/19/21.

| Scientific Name | Common Name | Group | ESA Listing |
|---|---|---|---|
| Ammodramus maritimus mirabilis | Cape Sable seaside sparrow | Birds | Endangered |
| Calidris canutus rufa | Red knot | Birds | Threatened |
| Charadrius melodus | Piping Plover | Birds | Threatened |
| Mycteria americana | Wood stork | Birds | Threatened |
| Myzomela cardinalis saffordi | Cardinal honey-eater | Birds | Resolved Taxon |
| Polyborus plancus audubonii | Audubon's crested caracara | Birds | Threatened |
| Rostrhamus sociabilis plumbeus | Everglade snail kite | Birds | Endangered |
| Sterna dougallii dougallii | Roseate tern | Birds | Threatened |
| Vermivora bachmanii | Bachman's warbler (=wood) | Birds | Endangered |
| Argythamnia blodgettii | Blodgett's silverbush | Flowering Plants | Threatened |
| Chamaecrista lineata keyensis | Big Pine partridge pea | Flowering Plants | Endangered |
| Chamaesyce deltoidea serpyllum | Wedge spurge | Flowering Plants | Endangered |
| Chamaesyce garberi | Garber's spurge | Flowering Plants | Threatened |
| Chromolaena frustrata | Cape Sable Thoroughwort | Flowering Plants | Endangered |
| Consolea corallicola | Florida semaphore Cactus | Flowering Plants | Endangered |
| Dalea carthagenensis floridana | Florida prairie-clover | Flowering Plants | Endangered |

| Scientific Name | Common Name | Group | ESA Listing |
|---|---|---|---|
| Digitaria pauciflora | Florida pineland crabgrass | Flowering Plants | Threatened |
| Indigofera mucronata var. keyensis | Florida indigo | Flowering Plants | Resolved Taxon |
| Linum arenicola | Sand flax | Flowering Plants | Endangered |
| Pilosocereus robinii | Key tree cactus | Flowering Plants | Endangered |
| Sideroxylon reclinatum ssp. austrofloridense | Everglades bully | Flowering Plants | Threatened |
| Anaea troglodyta floridalis | Florida leafwing Butterfly | Insects | Endangered |
| Cyclargus (=Hemiargus) thomasi bethunebakeri | Miami Blue Butterfly | Insects | Endangered |
| Danaus plexippus | monarch butterfly | Insects | Candidate |
| Heraclides aristodemus ponceanus | Schaus swallowtail butterfly | Insects | Endangered |
| Strymon acis bartrami | Bartram's hairstreak Butterfly | Insects | Endangered |
| Eumops floridanus | Florida bonneted bat | Mammals | Endangered |
| Neotoma floridana smalli | Key Largo woodrat | Mammals | Endangered |
| Odocoileus virginianus clavium | Key deer | Mammals | Endangered |
| Oryzomys palustris natator | Silver rice rat | Mammals | Endangered |
| Peromyscus gossypinus allapaticola | Key Largo cotton mouse | Mammals | Endangered |
| Puma (=Felis) concolor (all subsp. except coryi) | Puma (=mountain lion) | Mammals | Similarity of Appearance (Threatened) |
| Puma (=Felis) concolor coryi | Florida panther | Mammals | Endangered |
| Sylvilagus palustris hefneri | Lower Keys marsh rabbit | Mammals | Endangered |
| Trichechus manatus | West Indian Manatee | Mammals | Threatened |

| Scientific Name | Common Name | Group | ESA Listing |
|---|---|---|---|
| Alligator mississippiensis | American alligator | Reptiles | Similarity of Appearance (Threatened) |
| Caretta caretta | Loggerhead sea turtle | Reptiles | Threatened |
| Crocodylus acutus | American crocodile | Reptiles | Threatened |
| Dermochelys coriacea | Leatherback sea turtle | Reptiles | Endangered |
| Drymarchon corais couperi | Eastern indigo snake | Reptiles | Threatened |
| Eretmochelys imbricata | Hawksbill sea turtle | Reptiles | Endangered |
| Gopherus polyphemus | Gopher tortoise | Reptiles | Candidate |
| Orthalicus reses (not incl. nesodryas) | Stock Island tree snail | Snails | Threatened |

**Table A2.** Listed species in twelve California counties sorted by Group then Scientific Name. Information from [ HYPERLINK "https://www.fws.gov/endangered/" ] last accessed 8/12/21.

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ambystoma californiense | California tiger Salamander | Amphibians | Endangered | x | x | x | | | | x | | | x | x | x |
| Anaxyrus californicus | Arroyo (=arroyo southwestern) toad | Amphibians | Endangered | | | | x | x | x | | x | | | | |
| Anaxyrus canorus | Yosemite toad | Amphibians | Threatened | | x | | | | | | | | | | |
| Batrachoseps aridus | Desert slender salamander | Amphibians | Endangered | | | | | | x | | | | | | |
| Rana draytonii | California red-legged frog | Amphibians | Threatened | x | x | x | x | | | x | | x | x | | x |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rana muscosa | Mountain yellow-legged frog | Amphibians | Endangered | | x | | x | | x | | x | | | x | |
| Rana sierrae | Sierra Nevada Yellow-legged Frog | Amphibians | Endangered | | x | | | | | | | | | x | |
| Amphispiza belli clementeae | San Clemente sage sparrow | Birds | Threatened | | | | x | | | | | | | | |
| Brachyramphus marmoratus | Marbled murrelet | Birds | Threatened | x | | | x | x | | | | | | | |
| Centrocercus urophasianus | Greater sage-grouse | Birds | Resolved Taxon | | x | | | | | | | x | | x | |
| Charadrius nivosus nivosus | Western snowy plover | Birds | Threatened | x | | x | x | x | x | x | x | | | x | x |
| Coccyzus americanus | Yellow-billed Cuckoo | Birds | Threatened | x | x | x | x | | | x | | x | x | x | x |
| Coccyzus americanus ssp. occidentalis | No Common Name | Birds | Species of Concern | | x | | x | | | | x | | | | |
| Empidonax traillii extimus | Southwestern willow flycatcher | Birds | Endangered | | | | x | x | x | | x | | | x | |
| Gymnogyps californianus | California condor | Birds | Endangered | | x | x | x | | | | x | | | x | |
| Lanius ludovicianus mearnsi | San Clemente loggerhead shrike | Birds | Endangered | | | | x | | | | | | | | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phoebastria (=Diomedea) albatrus | Short-tailed albatross | Birds | Endangered | | | | x | x | | | | | | | |
| Pipilo crissalis eremophilus | Inyo California towhee | Birds | Threatened | | | | | | | | x | | | | |
| Polioptila californica californica | Coastal California gnatcatcher | Birds | Threatened | | | | x | x | x | | x | | | | |
| Rallus longirostris levipes | Light-footed clapper rail | Birds | Endangered | | | | x | x | | | | | | | |
| Rallus longirostris obsoletus | California clapper rail | Birds | Endangered | x | | | | | | x | | | | | |
| Rallus obsoletus [=longirostris] yumanensis | Yuma Ridgways (clapper) rail | Birds | Endangered | | | | | | x | | x | | | | |
| Sterna antillarum browni | California least tern | Birds | Endangered | x | | | x | x | | x | | | x | | |
| Strix occidentalis caurina | Northern spotted owl | Birds | Threatened | | | | | | | | | x | | | |
| Synthliboramphus hypoleucus | Xantus'sMurrelet | Birds | Resolved Taxon | | | | x | | | | | | | | |
| Vireo bellii pusillus | Least Bell's vireo | Birds | Endangered | | | | x | x | x | x | x | | x | x | x |
| Pinus albicaulis | Whitebark pine | Conifers and Cycads | Proposed Threatened | | x | | | | | | | x | | x | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Branchinecta conservatio | Conservancy fairy shrimp | Crustaceans | Endangered | x | x | x | x | | | x | | x | x | x | x |
| Branchinecta longiantenna | Longhorn fairy shrimp | Crustaceans | Endangered | x | | | | | | | | | | | |
| Branchinecta lynchi | Vernal pool fairy shrimp | Crustaceans | Threatened | x | x | x | x | | x | x | | x | x | x | x |
| Branchinecta sandiegonensis | San Diego fairy shrimp | Crustaceans | Endangered | | | | | x | | | | | | | |
| Lepidurus packardi | Vernal pool tadpole shrimp | Crustaceans | Endangered | x | x | x | | | | x | | x | x | x | x |
| Pacifastacus fortis | Shasta crayfish | Crustaceans | Endangered | | | | | | | | | x | | | |
| Streptocephalus woottoni | Riverside fairy shrimp | Crustaceans | Endangered | | | | x | x | x | | | | | | |
| Syncaris pacifica | California freshwater shrimp | Crustaceans | Endangered | | | | | | | | | | | | x |
| Catostomus santaanae | Santa Ana sucker | Fishes | Threatened | | | | x | x | x | | x | | | | |
| Cyprinodon macularius | Desert pupfish | Fishes | Endangered | | | | | | x | | | | | | |
| Cyprinodon radiosus | Owens pupfish | Fishes | Endangered | | x | | | | | | | | | x | |
| Eucyclogobius newberryi | Tidewater goby | Fishes | Endangered | x | | | x | x | | | | | | | |
| Gasterosteus aculeatus williamsoni | Unarmored threespine stickleback | Fishes | Endangered | | | | x | | | | x | | | | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gila bicolor ssp. mohavensis | Mohave tui chub | Fishes | Endangered | | | | | | | | x | | | | |
| Gila bicolor ssp. snyderi | Owens Tui Chub | Fishes | Endangered | | x | | | | | | | | | x | |
| Gila elegans | Bonytail | Fishes | Endangered | | | | | | | | x | | | | |
| Hypomesus transpacificus | Delta smelt | Fishes | Threatened | x | x | x | | | | x | | x | x | | x |
| Oncorhynchus aguabonita whitei | Little Kern golden trout | Fishes | Threatened | | | | | | | | | | | x | |
| Oncorhynchus clarkii henshawi | Lahontan cutthroat trout | Fishes | Threatened | | x | | | | | | | | | | |
| Oncorhynchus clarkii seleniris | Paiute cutthroat trout | Fishes | Threatened | | x | | | | | | | | | | |
| Ptychocheilus lucius | Colorado pikeminnow (=squawfish) | Fishes | Endangered | | | | | | | | x | | | | |
| Spirinchus thaleichthys | longfin smelt | Fishes | Candidate | x | x | | | | | x | | x | x | x | x |
| Xyrauchen texanus | Razorback sucker | Fishes | Endangered | | | | | | x | | x | | | | |
| Abronia alpina | Ramshaw Meadows sand-verbena | Flowering Plants | Resolved Taxon | | | | | | | | | | | x | |
| Acanthomintha obovata ssp. duttonii | San Mateo thornmint | Flowering Plants | Endangered | x | | | | | | | | | | | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acmispon dendroideus var. traskiae | San Clemente Island lotus (=broom) | Flowering Plants | Threatened | | | | x | | | | | | | | |
| Allium munzii | Munz's onion | Flowering Plants | Endangered | | | | | x | x | | | | | | |
| Ambrosia pumila | San Diego ambrosia | Flowering Plants | Endangered | | | | | | x | | | | | | |
| Amsinckia grandiflora | Large-flowered fiddleneck | Flowering Plants | Endangered | x | | | | | | | | | x | | |
| Arabis parishii | Parish's rock-cress | Flowering Plants | Species of Concern | x | | | | | | | | | | | |
| Arctostaphylos glandulosa ssp. crassifolia | Del Mar manzanita | Flowering Plants | Endangered | | | | | | x | | | | | | |
| Arctostaphylos myrtifolia | Ione manzanita | Flowering Plants | Threatened | | | | | | | x | | | | | |
| Arctostaphylos pallida | Pallid manzanita | Flowering Plants | Threatened | x | | | | | | | | | | | |
| Arenaria paludicola | Marsh Sandwort | Flowering Plants | Endangered | | x | x | x | | | | | | | | |
| Arenaria ursina | Bear Valley sandwort | Flowering Plants | Threatened | | | | | | | | x | | | | |
| Astragalus albens | Cushenbury milk-vetch | Flowering Plants | Endangered | | | | | | | | x | | | | |
| Astragalus brauntonii | Braunton's milk-vetch | Flowering Plants | Endangered | | | | x | x | x | | x | | | | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Astragalus jaegerianus | Lane Mountain milk-vetch | Flowering Plants | Endangered | | | | | | | | x | | | | |
| Astragalus lentiginosus var. coachellae | Coachella Valley milk-vetch | Flowering Plants | Endangered | | | | | | x | | x | | | | |
| Astragalus pycnostachyus var. lanosissimus | Ventura Marsh Milk-vetch | Flowering Plants | Endangered | | | | x | x | | | | | | | |
| Astragalus tener var. titi | Coastal dunes milk-vetch | Flowering Plants | Endangered | | | | x | | | | | | | | |
| Astragalus tricarinatus | Triple-ribbed milk-vetch | Flowering Plants | Endangered | | | | | | x | | x | | | | |
| Atriplex coronata var. notatior | San Jacinto Valley crownscale | Flowering Plants | Endangered | | | | | | x | | | | | | |
| Berberis nevinii | Nevin's barberry | Flowering Plants | Endangered | | | | x | | x | | x | | | | |
| Brodiaea filifolia | Thread-leaved brodiaea | Flowering Plants | Threatened | | | | x | x | x | | x | | | | |
| Brodiaea pallida | Chinese Camp brodiaea | Flowering Plants | Threatened | | | | | | | | | | x | | |
| Calochortus persistens | Siskiyou Mariposa lily | Flowering Plants | Resolved Taxon | | | | | | | | | x | | | |
| Calyptridium pulchellum | Mariposa pussypaws | Flowering Plants | Threatened | | x | | | | | | | | | | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Calystegia stebbinsii | Stebbins' morning-glory | Flowering Plants | Endangered | | | | | | | x | | | | | |
| Camissonia benitensis | San Benito evening-primrose | Flowering Plants | Threatened | | x | | | | | | | | | | |
| Castilleja campestris ssp. succulenta | Fleshy owl's-clover | Flowering Plants | Threatened | | x | | | | | x | | | x | | |
| Castilleja cinerea | Ash-grey paintbrush | Flowering Plants | Threatened | | | | | | | | x | | | | |
| Castilleja grisea | San Clemente Island Paintbrush | Flowering Plants | Threatened | | | | x | | | | | | | | |
| Caulanthus californicus | California jewelflower | Flowering Plants | Endangered | | x | x | | | | | | | | x | |
| Ceanothus ophiochilus | Vail Lake ceanothus | Flowering Plants | Threatened | | | | | | x | | | | | | |
| Ceanothus roderickii | Pine Hill ceanothus | Flowering Plants | Endangered | | | | | | | x | | | | | |
| Centaurium namophilum | Spring-loving centaury | Flowering Plants | Threatened | | | | | | | | x | | | | |
| Cercocarpus traskiae | Catalina Island mountain-mahogany | Flowering Plants | Endangered | | | | x | | | | | | | | |
| Chamaesyce hooveri | Hoover's spurge | Flowering Plants | Threatened | | | | | | | | | x | x | x | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chorizanthe parryi var. fernandina | San Fernando Valley Spineflower | Flowering Plants | Resolved Taxon | | | | x | | | | | | | | |
| Chorizanthe robusta var. robusta | Robust spineflower | Flowering Plants | Endangered | x | | | | | | | | | | | |
| Clarkia franciscana | Presidio clarkia | Flowering Plants | Endangered | x | | | | | | | | | | x | |
| Clarkia springvillensis | Springville clarkia | Flowering Plants | Threatened | | | | | | | | | | | x | |
| Cordylanthus maritimus ssp. maritimus | Salt marsh bird's-beak | Flowering Plants | Endangered | | | | x | x | | | | | | | |
| Cordylanthus mollis ssp. mollis | Soft bird's-beak | Flowering Plants | Endangered | | | | | | | x | | | | | |
| Cordylanthus palmatus | Palmate-bracted bird's beak | Flowering Plants | Endangered | x | x | | | | | x | | | | | x |
| Delphinium variegatum ssp. kinkiense | San Clemente Island larkspur | Flowering Plants | Endangered | | | | x | | | | | | | | |
| Dodecahema leptoceras | Slender-horned spineflower | Flowering Plants | Endangered | | | | x | x | x | | x | | | | |
| Dudleya abramsii ssp. parva | Conejo dudleya | Flowering Plants | Threatened | | | | x | | | | | | | | |
| Dudleya cymosa ssp. marcescens | Marcescent dudleya | Flowering Plants | Threatened | | | | x | | | | | | | | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dudleya cymosa ssp. ovatifolia | Santa Monica Mountains dudleyea | Flowering Plants | Threatened | | | | x | x | x | | x | | | | |
| Dudleya setchellii | Santa Clara Valley dudleya | Flowering Plants | Threatened | | | | | | | | | | x | | |
| Dudleya stolonifera | Laguna Beach liveforever | Flowering Plants | Threatened | | | | | x | | | | | | | |
| Dudleya verityi | Verity's dudleya | Flowering Plants | Threatened | | | | x | | | | | | | | |
| Eremalche kernensis | Kern mallow | Flowering Plants | Endangered | | | | | | | | | | | x | |
| Eriastrum densifolium ssp. sanctorum | Santa Ana River woolly-star | Flowering Plants | Endangered | | | | | | x | | x | | | | |
| Erigeron parishii | Parish's daisy | Flowering Plants | Threatened | | | | | | x | | x | | | | |
| Eriogonum apricum (incl. var. prostratum) | Ione (incl. Irish Hill) buckwheat | Flowering Plants | Endangered | | | | | | | x | | | | | |
| Eriogonum kennedyi var. austromontanum | Southern mountain wild-buckwheat | Flowering Plants | Threatened | | | | | | | | x | | | | |
| Eriogonum ovalifolium var. vineum | Cushenbury buckwheat | Flowering Plants | Endangered | | | | | | | | x | | | | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Eryngium aristulatum var. parishii | San Diego button-celery | Flowering Plants | Endangered | | | | | x | x | | | | | | |
| Erysimum capitatum var. angustatum | Contra Costa wallflower | Flowering Plants | Endangered | | | | | | | x | | | | | |
| Fremontodendron californicum ssp. decumbens | Pine Hill flannelbush | Flowering Plants | Endangered | | | | | | | x | | | | | |
| Galium californicum ssp. sierrae | El Dorado bedstraw | Flowering Plants | Endangered | | | | | | | x | | | | | |
| Helianthemum greenei | Island rush-rose | Flowering Plants | Threatened | | | | x | | | | | | | | |
| Holocarpha macradenia | Santa Cruz tarplant | Flowering Plants | Threatened | x | | | | | | | | | | | |
| Lasthenia burkei | Burke's goldfields | Flowering Plants | Endangered | | | | | | | | | | | | x |
| Lasthenia conjugens | Contra Costa goldfields | Flowering Plants | Endangered | x | | | | | | | | | | | |
| Lesquerella kingii ssp. bernardina | San Bernardino Mountains bladderpod | Flowering Plants | Endangered | | | | | | | | x | | | | |
| Lithophragma maximum | San Clemente Island woodland-star | Flowering Plants | Endangered | | | | x | | | | | | | | |

| Scientific Name | Common Name | Group | ESA Listing | Alameda | Fresno | Kings | Los Angeles | Orange | Riverside | Sacramento | San Bernardino | Shasta | Stanislaus | Tulare | Yolo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Malacothamnus clementinus | San Clemente Island bush-mallow | Flowering Plants | Endangered | | | | x | | | | | | | | |
| Mimulus shevockii | Kelso Creek monkey-flower | Flowering Plants | Resolved Taxon | | | | | | | | | | | x | |
| Monolopia (=Lembertia) congdonii | San Joaquin wooly-threads | Flowering Plants | Endangered | | x | x | | | | | | | | | |
| Navarretia fossalis | Spreading navarretia | Flowering Plants | Threatened | | | | x | | x | | | | | | |
| Neostapfia colusana | Colusa grass | Flowering Plants | Threatened | | | | | | | x | | | x | | x |
| Neviusia cliftonii | Shasta snow-wreath | Flowering Plants | Under Review | | | | | | | | | x | | | |
| Oenothera deltoides ssp. howellii | Antioch Dunes evening-primrose | Flowering Plants | Endangered | | | | | | | x | | | | | |
| Orcuttia californica | California Orcutt grass | Flowering Plants | Endangered | | | | x | | x | | | | | | |
| Orcuttia inaequalis | San Joaquin Orcutt grass | Flowering Plants | Threatened | | x | | | | | | | | x | x | |
| Orcuttia pilosa | Hairy Orcutt grass | Flowering Plants | Endangered | | x | | | | | | | | x | | |
| Orcuttia tenuis | Slender Orcutt grass | Flowering Plants | Threatened | | | | | | | x | | x | | | |
| Orcuttia viscida | Sacramento Orcutt grass | Flowering Plants | Endangered | | | | | | | x | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oxytheca parishii var. goodmaniana | Cushenbury oxytheca | Flowering Plants | Endangered | | | | | | | | x | | | | |
| Pentachaeta lyonii | Lyon's pentachaeta | Flowering Plants | Endangered | | | | x | | | | | | | | |
| Phacelia stellaris | Brand's phacelia | Flowering Plants | Resolved Taxon | | | | x | | | | | | | | |
| Poa atropurpurea | San Bernardino bluegrass | Flowering Plants | Endangered | | | | | | | | x | | | | |
| Pseudobahia bahiifolia | Hartweg's golden sunburst | Flowering Plants | Endangered | | x | | | | | | | | x | | |
| Pseudobahia peirsonii | San Joaquin adobe sunburst | Flowering Plants | Threatened | | x | | | | | | | | | x | |
| Rorippa gambellii | Gambel's watercress | Flowering Plants | Endangered | | | | x | | x | | x | | | | |
| Senecio layneae | Layne's butterweed | Flowering Plants | Threatened | | | | | | | x | | | | | |
| Sibara filifolia | Santa Cruz Island rockcress | Flowering Plants | Endangered | | | | x | | | | | | | | |
| Sidalcea keckii | Keck's Checker-mallow | Flowering Plants | Endangered | | x | | | | | | | | | x | x |
| Sidalcea pedata | Pedate checker-mallow | Flowering Plants | Endangered | | | | | | | | x | | | | |
| Suaeda californica | California seablite | Flowering Plants | Endangered | x | | | | | | | | | | | |
| Taraxacum californicum | California taraxacum | Flowering Plants | Endangered | | | | | | | | x | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Thelypodium stenopetalum | Slender-petaled mustard | Flowering Plants | Endangered | | | | | | | | x | | | | |
| Thysanocarpus conchuliferus | Santa Cruz Island fringepod | Flowering Plants | Endangered | | | | x | | | | | | | | |
| Trichostema austromontanum ssp. compactum | Hidden Lake bluecurls | Flowering Plants | Recovery | | | | | | x | | | | | | |
| Tuctoria greenei | Greene's tuctoria | Flowering Plants | Endangered | | x | | | | | | | x | x | x | |
| Tuctoria mucronata | Solano grass | Flowering Plants | Endangered | | | | | | | | | | | | x |
| Verbena californica | Red Hills vervain | Flowering Plants | Threatened | | | | | | | | | | x | | |
| Verbesina dissita | Big-leaved crownbeard | Flowering Plants | Threatened | | | | | x | | | | | | | |
| Apodemia mormo langei | Lange's metalmark butterfly | Insects | Endangered | | | | | | | x | | | | | |
| Bombus franklini | Franklin's bumblebee | Insects | Proposed Endangered | | | | | | | | | x | | | |
| Danaus plexippus | monarch butterfly | Insects | Candidate | x | x | x | x | x | x | x | x | x | x | x | x |
| Desmocerus californicus dimorphus | Valley elderberry longhorn beetle | Insects | Threatened | x | x | | | | | x | | x | x | | x |
| Dinacoma caseyi | Casey's June Beetle | Insects | Endangered | | | | | | x | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Euphilotes battoides allyni | El Segundo blue butterfly | Insects | Endangered | | | | x | | | | | | | | |
| Euphydryas editha quino (=E. e. wrighti) | Quino checkerspot butterfly | Insects | Endangered | | | | | x | x | | x | | | | |
| Glaucopsyche lygdamus palosverdesensis | Palos Verdes blue butterfly | Insects | Endangered | | | | x | | | | | | | | |
| Rhaphiomidas terminatus abdominalis | Delhi Sands flower-loving fly | Insects | Endangered | | | | | | x | | x | | | | |
| Canis lupus | Gray wolf | Mammals | Recovery | | | | | | | | | x | | | |
| Dipodomys ingens | Giant kangaroo rat | Mammals | Endangered | | x | x | | | | | | | | x | |
| Dipodomys merriami parvus | San Bernardino Merriam's kangaroo rat | Mammals | Endangered | | | | x | | x | | x | | | | |
| Dipodomys nitratoides exilis | Fresno kangaroo rat | Mammals | Endangered | | x | x | | | | | | | x | x | |
| Dipodomys nitratoides nitratoides | Tipton kangaroo rat | Mammals | Endangered | | x | x | | | | | | | | x | |
| Dipodomys stephensi (incl. D. cascus) | Stephens' kangaroo rat | Mammals | Endangered | | | | | x | x | | x | | | | |
| Gulo gulo luscus | North American wolverine | Mammals | Resolved Taxon | | x | | | | | | | | | x | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Microtus californicus scirpensis | Amargosa vole | Mammals | Endangered | | x | | | | | | x | | | x | |
| Neotoma fuscipes riparia | Riparian woodrat (=San Joaquin Valley) | Mammals | Endangered | | | | | | | | | | x | | |
| Ovis canadensis nelsoni | Peninsular bighorn sheep | Mammals | Endangered | | | | | | x | | | | | | |
| Ovis canadensis sierrae | Sierra Nevada bighorn sheep | Mammals | Endangered | | x | | | | | | | | | x | |
| Pekania pennanti | Fisher | Mammals | Resolved Taxon | | | | | | | | | x | | | |
| Perognathus longimembris pacificus | Pacific pocket mouse | Mammals | Endangered | | | | x | x | | | | | | | |
| Reithrodontomys raviventris | Salt marsh harvest mouse | Mammals | Endangered | x | | | | | | | | | | | |
| Spermophilus mohavensis | Mohave ground squirrel | Mammals | Resolved Taxon | | | | x | | | | x | | | | |
| Spermophilus tereticaudus chlorus | Palm Springs round-tailed ground (=Coachella Valley) Squirrel | Mammals | Resolved Taxon | | | | | | x | | x | | | | |
| Sylvilagus bachmani riparius | Riparian brush rabbit | Mammals | Endangered | | | | | | | x | | | x | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Urocyon littoralis catalinae | Santa Catalina Island Fox | Mammals | Threatened | | | | x | | | | | | | | |
| Vulpes macrotis mutica | San Joaquin kit fox | Mammals | Endangered | x | x | x | x | | | | | | x | x | |
| Vulpes velox | Swift fox | Mammals | Resolved Taxon | | x | x | | | | | | | | x | |
| Dermochelys coriacea | Leatherback sea turtle | Reptiles | Endangered | | x | | x | x | | | | | | | |
| Gambelia silus | Blunt-nosed leopard lizard | Reptiles | Endangered | | x | x | x | | | | | | x | | |
| Gopherus agassizii | Desert tortoise | Reptiles | Threatened | | | | x | | x | | x | | | x | |
| Lepidochelys olivacea | Olive ridley sea turtle | Reptiles | Threatened | | x | | x | x | | | | | | | |
| Masticophis lateralis euryxanthus | Alameda whipsnake (=striped racer) | Reptiles | Threatened | x | | | | | | | | | x | | |
| Thamnophis gigas | Giant garter snake | Reptiles | Threatened | x | x | x | | | | x | | | x | x | x |
| Thamnophis sirtalis tetrataenia | San Francisco garter snake | Reptiles | Endangered | x | | | | | | | | | | | |
| Uma inornata | Coachella Valley fringe-toed lizard | Reptiles | Threatened | | | | | | x | | | | | | |
| Xantusia riversiana | Island night lizard | Reptiles | Recovery | | | | x | | | | | | | | |
| Fluminicola seminalis | Nugget pebblesnail | Snails | Resolved Taxon | | | | | | | | | x | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lyogyrus spc | Canary duskysnail | Snails | Resolved Taxon | | | | | | | | | x | | | |
| Trilobopsis roperi | Shasta chaparral | Snails | Under Review | | | | | | | | | x | | | |
| Vespericola shasta | Shasta hesperian | Snails | Under Review | | | | | | | | | x | | | |
| Vorticifex sp | Knobby Rams-horn | Snails | Resolved Taxon | | | | | | | | | x | | | |